

Application Parameters Affecting Field Efficacy of *Beauveria bassiana* Foliar Treatments against Colorado Potato Beetle *Leptinotarsa decemlineata*¹

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The effects of various spray-application parameters on the efficacy of *Beauveria bassiana* foliar treatments against *Leptinotarsa decemlineata* larvae were evaluated during three field seasons. Treatments were applied to small plots (6 rows \times 7–12 m) using a CO₂-powered backpack hydraulic sprayer with nozzles affixed to lateral drop tubes and directed upward at a 45° angle to target ventral leaf surfaces. The sprayer delivered 280 liters/ha at 3.45 bar. Three rates (1.25, 2.5, and 5 \times 10¹³ conidia/ha) of an emulsifiable oil formulation of *B. bassiana* and two spray intervals (3–4 vs 6–8 days) were tested. Three applications at the medium and high rates made at 3- to 4-day intervals produced low to moderate levels of control (43–65%). The low rate and applications at weekly intervals were less effective. A wettable powder (WP) formulation was also compared to the oil formulation. Three applications at the medium rate were made at 3- to 4-day intervals. During one field season, rain commenced soon after two of the three initial applications. Under these conditions, the oil formulation was significantly more effective than the WP formulation (65% vs 8% control), suggesting greater rainfastness of the oil formulation. Tests with the oil formulation also compared applications from a sprayer configured with drop tubes as described above to those from a sprayer configured with nozzles directed to spray downward from 30 cm above the canopy. The sprayer with nozzles attached solely on drop tubes deposited an average of 752 conidia/mm² on the upper surfaces of the leaves and 482 conidia/mm² (39%) on the lower surfaces, whereas the alternative sprayer deposited 1062 conidia/mm² onto the upper surfaces and only 50 conidia/mm² (<5%) onto the leaf undersides. Applica-

tions from below canopy provided greater control of larvae than applications from above canopy when initiated against early instars. Applications initiated against late-instar larvae failed to provide useful control of larvae; however, nearly all treatments applied against late instars, including sprays at weekly intervals and from above canopy, produced significant reductions (53–84%) in populations of first-generation adult beetles.

Key Words: *Beauveria bassiana*; entomopathogenic fungus; *Leptinotarsa decemlineata*; Colorado potato beetle; application methods; field efficacy; microbial control.

INTRODUCTION

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is a key pest of potatoes worldwide. Uncontrolled populations are capable of completely defoliating a potato crop and causing total yield losses. Conventional management systems rely almost exclusively on applications of synthetic chemical insecticides; however, this insect exhibits an exceptional capacity to rapidly develop resistance to chemical insecticides (Gauthier *et al.*, 1981), and alternative control agents are needed for sustainable integrated management programs.

The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin is an important natural enemy of the Colorado potato beetle. High levels of natural infection are most commonly encountered in populations of prepupae, pupae, and adults in the soil, but larval stages are also highly susceptible, and *B. bassiana* has a long history of development as a larvicide for foliage protection. Results, however, have been highly variable (Blonska, 1957; Lappa, 1978; Fargues *et al.*, 1980; Roberts *et al.*, 1981; Galaini, 1984; Campbell *et al.*, 1985; Hajek *et al.*, 1987; Anderson *et al.*, 1988; Poprawski *et al.*, 1997; Lacey *et al.*, 1999), and foliar applica-

¹ Product names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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tions generally have not provided commercially acceptable control.

Beauveria bassiana is more virulent against early- versus late-instar larvae of Colorado potato beetle (Blonska, 1957; Fargues, 1972). A model developed by T. Larkin, S. Galaini, and R. Carruthers (unpublished in Galaini, 1984) predicted that *B. bassiana* would not provide effective control of Colorado potato beetle larvae when applied at weekly intervals. The reason for this lack of control is because a 1-week interval under normal field conditions would permit larval eclosion and development to a more resistant stage (third instar). Galaini (1984) demonstrated improved efficacy from applications made on 3- to 4-day intervals compared to 7-day intervals, but these tests were conducted during different field seasons. Effects of changing spray intervals have not been extensively examined in directly comparable treatments.

Because *B. bassiana* is most virulent against early-instar larvae, and because early instars feed primarily on the ventral leaf surfaces, we hypothesized that targeting of the leaf undersides would enhance the larvicidal efficacy of foliar sprays. Conidia of *B. bassiana* are also highly susceptible to solar radiation (Galaini, 1984; Inglis *et al.*, 1995a). Development of effective, economical sunscreens formulations has been pursued for many years, but with only limited success. An alternative strategy for protecting fungi from UV degradation is targeting of spray applications to ensure deposition in niches frequented by the insect host but shaded from the sun. The ventral surfaces of potato foliage represent such a niche.

Studies in recent years, primarily laboratory tests of *Metarhizium anisopliae* (Metschnikoff) Sorokin var. *acridum* Driver & Milner (formerly identified as *Metarhizium flavoviride* Gams & Rozsypal), have indicated that formulation of hyphomycete conidia in oil increases efficacy (Prior *et al.*, 1988; Bateman *et al.*, 1993; Milner *et al.*, 1997; Fargues *et al.*, 1997b). Putative mechanisms for this increased efficacy include the greater adhesion of oils to the lipophilic insect cuticle and spreading of oil droplets impacting the cuticle. Spreading of oil on the cuticle may carry conidia into niches on the host cuticle (e.g., intersegmental folds) that provide moisture for germination and protection from solar radiation (Ibrahim *et al.*, 1999). However, most reports of efficacy enhancement from oils are from laboratory studies, and even in these, the reported effect is not great, especially at rates comparable to those recommended for field applications (see reviews by Wraight and Carruthers, 1999; Wraight *et al.*, 2001). The impact of oil formulation on fungal efficacy under field conditions has been the subject of only a few investigations. Even fewer studies have examined effects of formulation in emulsifiable oils.

Commercial formulations of *B. bassiana* have been developed and registered worldwide. Mycotrol, devel-

oped by Mycotech Corp. of Butte, Montana (now Emerald BioAgriculture Corp.), is based on a strain of *B. bassiana* (GHA) that is pathogenic against a broad range of insect pests, including the Colorado potato beetle. The standard label rate of this product (corresponding to 2.5×10^{13} conidia/ha) is currently marketed at a cost exceeding that of many highly effective broad-spectrum synthetic insecticides. Demonstrated efficacy of a lower field rate would have an important impact on the economics of *B. bassiana* use for Colorado potato beetle control.

This study was undertaken primarily to assess the importance of various spray-application parameters affecting larvicidal efficacy of *B. bassiana*, with the ultimate objective being development of a delivery system providing effective and consistent control at an acceptable cost. In view of the problems outlined above, the field studies reported here were designed to assess four factors: (1) application rates from 1.25 to 5×10^{13} conidia/ha, (2) spray intervals of 3–4 days versus 6–8 days, (3) spray boom configurations targeting dorsal versus ventral leaf surfaces, and (4) formulation of conidia as an oil-based emulsifiable suspension (ES) versus a wettable powder (WP).

MATERIALS AND METHODS

The studies were conducted in small, replicated field plots on the H. C. Thompson Vegetable Crops Research Farm of the Cornell University Department of Entomology in Freeville, New York, and were the result of an informal collaboration between the USDA and Mycotech Corporation.

Fungal Preparations

The strain of *B. bassiana* used was derived from strain ARSEF 201 (originally from *Diabrotica undecimpunctata* Mannerheim collected in Corvallis, OR). Single-conidium isolations from strain 201 produced colonies with variable morphologies, and from these, a type with desirable growth and sporulation characteristics was selected (C. A. Bradley, personal communication). Subsequently, in 1991, this fungus was passaged through grasshoppers inoculated in laboratory bioassays (Bradley *et al.*, 1999) and then reisolated in 1994 from a laboratory-inoculated *Bemisia argentifolii* Bellows & Perring nymph (S. P. Wraight, unpublished). The whitefly reisolates (designated strain BB-726 during development) was registered by Mycotech Corp. as strain GHA and comprises the active ingredient in the commercial products Mycotrol and BotaniGard. This fungus was recently deposited as strain 6444 in the Agricultural Research Service Collection of Entomopathogenic Fungi at Ithaca, New York.

Fungal formulations were produced at the Mycotech laboratory in Butte, Montana, utilizing proprietary

methods and ingredients (see Bradley *et al.*, 1992). For the field tests, the fungus was formulated as an oil-based emulsifiable suspension (Mycotrol ES) containing 2.1×10^{10} conidia/ml and as a clay-based wettable powder (Mycotrol 22WP) containing 4.4×10^{10} conidia/g. All preparations were stored at 4°C and retained high viability (>93%) for the duration of the study. Concentrations in the unformulated conidial powders used to prepare the WP and ES formulations were determined by preparing aqueous suspensions (1–3 mg powder/ml) and counting conidia at 400× magnification in standard (improved Neubauer) hemacytometer chambers. Viability of conidia was determined by direct observation of conidia (400× magnification) plated on agar containing yeast extract (0.5%) and incubated 16–18 h at 25°C. All conidia with visible germ tubes were scored as viable. Amounts of conidial powder necessary to achieve the desired rates (viable conidia/ha) in a specific spray volume were determined from these conidial concentration and viability data.

Chemical Pesticides

The recent appearance in central New York of hypervirulent strains of the late blight pathogen *Phytophthora infestans* (Montagne) de Bary made prophylactic applications of fungicides mandatory (especially to protect experimental crops on adjacent fields). Fungicides were applied when weather conditions favored late blight development. Materials and rates used included Bravo 720 or Bravo Weather Stik (Zeneca Ag Products, Wilmington, DE) at 1.68 kg (0.91 kg chlorothalonil)/ha; Penncozeb DF (Elf Atochem, Philadelphia, PA) at 2.24 kg (1.68 kg maneb)/ha; Dithane M-45 (Rohm and Haas, Philadelphia, PA) at 2.24 kg (1.79 kg mancozeb)/ha; Manzate 200 (DuPont Agricultural Products, Wilmington, DE) at 2.24 kg (1.68 kg mancozeb)/ha; and Ridomil MZ-58 (Ciba, Greensboro, NC) at 2.24 kg (0.22 kg metalaxyl + 1.08 kg mancozeb)/ha. Monitor 4 (Bayer, Kansas City, MO) was applied at 1.17 liters (0.56 kg methamidophos)/ha for control of potato leafhopper *Empoasca fabae* (Harris).

Fungicides and insecticides were applied using a conventional hydraulic sprayer configured for above-canopy sprays, with TeeJet (Spraying Systems Co., Wheaton, IL) TJ60-8006EVS spray nozzles spaced 50.8 cm apart. Spray volume was 170 liters/ha applied at a pressure of 3.1 bar.

Field Tests

1997. Potatoes ("Atlantic") were planted 27 May in plots measuring 6 rows × 12.2 m with rows spaced 86 cm. Each treatment was applied to four replicate plots. Applications were made using a backpack, single-row, CO₂-powered, hydraulic sprayer (R & D Sprayers, Opelousas, LA) fitted with three TeeJet TXVS-6 hollow-cone spray nozzles. The center nozzle was mounted on

a fixed tube and directed straight downward from 15 to 20 cm above the crop canopy. Each of the two lateral nozzles was mounted on a swivel affixed to a drop tube; these nozzles were carried 15–20 cm above the ground and were directed at a 45° angle to spray upward into the potato canopy (maximizing coverage of ventral leaf surfaces). The sprayer delivered 280 liters/ha at a pressure of 3.45 bar. Three rates (1.25, 2.5, and 5×10^{13} conidia/ha) of the oil-based ES formulation of *B. bassiana* strain GHA (Mycotrol ES) and two spray intervals were tested. Four applications at 3- to 4-day intervals (1, 5, 8, and 11 July) and two weekly applications (1 and 8 July) were made. A wettable powder formulation (Mycotrol 22WP) was also compared to the ES formulation. Four sprays at the medium rate of 2.5×10^{13} conidia/ha were applied at 3- to 4-day intervals. Tests with the ES formulation also compared applications from a sprayer configured as described above (with lateral drop tubes) to those from a sprayer configured with all three nozzles directed to spray straight downward from ~30 cm above the crop canopy. A summary of the spray treatments and controls is presented in Table 1. Penncozeb DF was applied for late blight control on 27 June; 10, 15, and 25 July; and 6, 15, 22, and 28 August; and Monitor 4 was applied against leafhoppers on 27 June.

1998. Potatoes ("Allegheny") were planted 15 May in plots measuring 6 rows × 9.1 m with rows spaced 86 cm. Treatments (Table 1) were applied as in 1997 using the same sprayer, but with TXVS-8 nozzles fitted only on the lateral drop tubes (the central nozzle was removed). Each treatment was applied to five replicate plots. Three applications at 3- to 4-day intervals (1, 5, and 9 July) and two weekly applications (1 and 9 July) were applied. Fungicide applications included Manzate 200 on 2, 15, 21, and 27 July and 3 and 21 August, Bravo 720 on 11 August, and Ridomil MZ-58 on 11 September. Monitor 4 was applied for leafhopper control on 15 July.

1999. Potatoes ("Salem") were planted 17 May in plots measuring 6 rows × 7.6 m with rows spaced 86 cm. Treatments (Table 1) were applied as in 1998. Each treatment was applied to six replicate plots. Three applications at 3- to 4-day intervals (29 June and 2 and 5 July) and two weekly applications (29 June and 5 July) were made. Fungicide applications included Dithane M-45 on 12 July and Bravo Weather Stik on 20 and 29 July and 5 and 12 August.

Conidia sampling. Conidial deposition was monitored by attaching 22-mm square plastic coverslips to the upper and/or lower surfaces of the plant foliage in selected treatments (see Table 3). Coverslips were pierced through the center by a pin which was passed through the leaf and embedded in a small (1-cm) cube of dense polyethylene foam. Leaves selected for sampling were those in the outer mid- to upper canopy.

TABLE 1

Treatments Applied to Research Plots of Potatoes Infested with Colorado Potato Beetles during Three Consecutive Field Seasons

| Treatment ^a | Code | Year applied |
|--------------------------------------------------------------------------------------------------|--------------------|------------------|
| Untreated control | Untreated | 1997, 1998, 1999 |
| ES formulation carrier control: Three or four below-canopy applications at 3- to 4-day intervals | ES carrier control | 1997, 1998, 1999 |
| WP formulation carrier control: three or four below-canopy applications at 3- to 4-day intervals | WP carrier control | 1997, 1998 |
| ES; low rate: three or four below-canopy applications at 3- to 4-day intervals | ES-L-BC-3d | 1997, 1998 |
| ES; medium rate: three or four below-canopy applications at 3- to 4-day intervals | ES-M-BC-3d | 1997, 1998, 1999 |
| ES; high rate: three or four below-canopy applications at 3- to 4-day intervals | ES-H-BC-3d | 1997, 1998 |
| ES; low rate: two below-canopy applications made 7–8 days apart | ES-L-BC-7d | 1997, 1998 |
| ES; medium rate: two below-canopy applications made 6–8 days apart | ES-M-BC-7d | 1997, 1998, 1999 |
| ES; high rate: two below-canopy applications made 7–8 days apart | ES-H-BC-7d | 1997, 1998 |
| ES; medium rate: three or four above-canopy applications at 3- to 4-day intervals | ES-M-AC-3d | 1997, 1998, 1999 |
| ES; medium rate: two above-canopy applications made 7–8 days apart | ES-M-AC-7d | 1997, 1998 |
| WP; medium rate: three or four below-canopy applications at 3- to 4-day intervals | WP-M-BC-3d | 1997, 1998, 1999 |
| WP; medium rate: two below-canopy applications made 7–8 days apart | WP-M-BC-7d | 1997, 1998 |

^a Application rates of an emulsifiable suspension (ES) and wettable powder (WP) formulation of *Beauveria bassiana*: low = 1.25×10^{12} conidia/ha; medium = 1×10^{13} conidia/ha; high = 2×10^{13} conidia/ha. Carrier controls: ES formulation blank was applied at the high rate (2.34 liters/ha) in 1997 and 1998 and at the medium rate (1.17 liters/ha) in 1999; WP formulation blank was applied at the medium rate (0.56 kg/ha) in 1997 and 1998.

Following spray application, the coverslips were allowed to dry before collection and then stored and counted as time allowed. On each application date, a total of 25 coverslips were attached to dorsal surfaces, and an equal number to ventral surfaces, of leaves on randomly selected plants in two of the replicate plots of each treatment (12–13 coverslips/plot/leaf surface). Additional replication was achieved by sampling on a minimum of 3 spray dates. For quantification of conidia, the coverslips were mounted in a drop of lactic acid (85%) containing acid fuchsin (1 mg/ml) and viewed at 400 \times magnification. Enumeration was achieved using the protocol described by Wraight *et al.* (1998).

Viability of conidia was checked routinely during all field trials by collecting samples of the residual spray suspensions from the sprayer reservoirs and incubating on yeast extract agar as described above.

Environmental monitoring. Rainfall was monitored using portable electronic data loggers (Omnidata International, Inc., Logan, UT) maintained in the test field. Hourly records of air temperature, relative humidity, and solar radiation were obtained from a Cornell University weather station located approximately 13 km from the field site.

Insect sampling. Leaf samples were collected within 1 day prior to the initial application and at irregular intervals of 1–7 days thereafter. On each sample date, 10 stems (20–25 cm in length) were collected from random locations within each replicate plot. All collected leaves were held in plastic bags at 4°C prior to processing. All live larvae and adults on

the 10 stems collected from each plot were counted; larvae were categorized as early (first and second) or late (third and fourth) instars. The 1997 and 1999 larval populations were sampled for approximately 2 weeks after the initial application. Because of a weather-related delay in initiating the spray program in 1998, and because few treatment effects were observed within 2 weeks, sampling was extended over a 5-week period.

Defoliation and yield determinations. Defoliation was assessed each season approximately 2 weeks after the initial application. Individual potato plants were examined at 15 randomized locations in each plot in 1997 and 1998 and at 10 locations in 1999, and the level of defoliation was estimated to the nearest 10%. In late September, 1999, all potatoes were harvested from each plot and weighed. Yield was expressed as total kilograms potatoes per meter of row. Low beetle populations inflicted minor damage in 1997 and 1998, and yields were not assessed.

Statistical analyses. All plots in each test were arranged in a randomized, complete block design with one replicate per block. Two-way ANOVAs with planned orthogonal means comparisons were conducted using the JMP statistical software (SAS Institute, 1995); treatment means from each trial were compared to the controls using Dunnett's procedure (Zar, 1999). Colorado potato beetle larval population responses to the multiple spray applications were expressed gradually over extended periods of time, and a simple approach was adopted for evaluating differences in treatment effects. Analyses were conducted on

means derived from the grand total larvae recorded on all stems collected over a specified period of time from each plot. Treatment means were thus based on four to six values (one from each block) regardless of the number of sample dates included. The logarithmic transformation was applied to all beetle numbers and potato yields (weights) subjected to ANOVA. Proportion defoliation estimates were subjected to standard arcsine transformation (Zar, 1999). Conidial counts from the coverslip samples were square-root-transformed prior to two-way ANOVA (including application dates and treatments as main effects). As with the beetle counts, all ANOVAs of the defoliation and yield data were conducted on mean values from the four to six replicate plots. ANOVAs of the conidial counts were conducted on the mean values from the two replicate plots sampled during three consecutive applications.

RESULTS

Larval populations were low during the 1997 and 1998 field seasons, with densities in the controls never exceeding 3 larvae/stem. This, combined with the tendency of Colorado potato beetle populations to be highly aggregated, resulted in high variability of the sample data, as reflected in the large standard errors (Figs. 1–4). Populations at the site increased during the study to approximately 5 larvae/stem in 1999.

Preliminary analyses revealed no consistent significant differences (from two or more successive samples) among larval populations in the untreated and spray-carrier controls during each field season (Figs. 1, 3, and 5). Probability (P) values from direct orthogonal comparisons of the controls in 1997, 1998, and 1999 were, in all cases, >0.50 (Tables 2, 4, and 5). Sample data from each year's control treatments were consequently combined to provide a single estimate of natural (untreated) population density to which each treatment was compared.

In the following presentation and discussion of results, all references to control of larvae or larval populations refer to the first-generation larvae inhabiting the crop canopy. For simplicity, treatments in which multiple sprays were applied at 3- or 4-day intervals and those in which two sprays were applied 6–8 days apart are referred to as having received applications at 3- versus 7-day (or weekly) intervals.

1997 Results

Initial applications were made on 1 July, during first-generation egg hatch. A sample collected on the previous day revealed that the larval population comprised 94.7% early instars. Comparison of the ES treatment with the controls indicated an effect within 6 days (Fig. 1), and the analysis was conducted on total numbers of larvae collected on five occasions between days 6 and 16 posttreatment (Table 2).

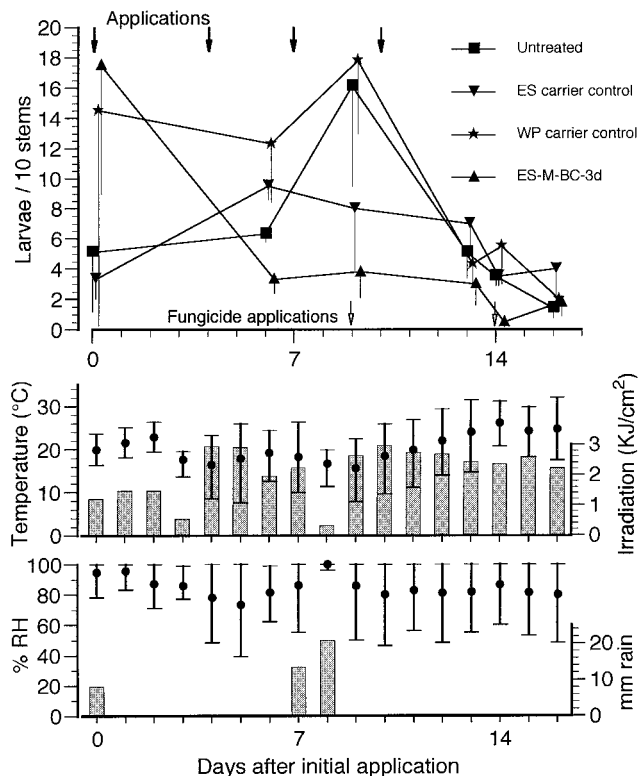


FIG. 1. Trends in Colorado potato beetle larval populations during a program of multiple spray applications of *Beauveria bassiana* conidia during a 1997 field trial; vertical lines represent standard errors of means ($n = 4$). Environmental data include temperature and relative humidity (daily means, maxima, and minima) and daily rainfall and solar irradiation. The initial application was made on 1 July. Explanation of treatment codes is presented under Table 1.

Analysis of the conidial-deposition (coverslip) samples indicated no significant differences among the numbers of conidia deposited on the dorsal leaf surfaces over the three spray dates [$F(2, 9) = 3.8$; $P = 0.07$]; there was no indication of a treatment \times date interaction ($P = 0.43$). In contrast, significant among-date differences were detected in the spore depositions on ventral surfaces [$F(2, 9) = 12.1$; $P = 0.003$]; however, in this case, the treatment \times date interaction was significant ($P = 0.008$). Sprays of the ES formulation made at the medium rate using the drop-tube configuration deposited an average of 1137 and 259 conidia/mm² on the dorsal and ventral leaf surfaces, respectively (Table 3). The rate of 259 conidia/mm² represented 19% of the total deposition (1396 conidia/mm² on both surfaces combined). Applications of the comparable treatment from above canopy deposited fewer [$F(1, 6) = 11.2$; $P = 0.016$] total conidia (1082 conidia/mm²) and only 28 conidia/mm² ($<3\%$) on the ventral leaf surfaces. Total depositions of conidia from the comparable ES and WP applications (medium rate applied from below canopy) did not differ significantly [$F(1, 6) = 2.5$; $P = 0.17$], and there was no interac-

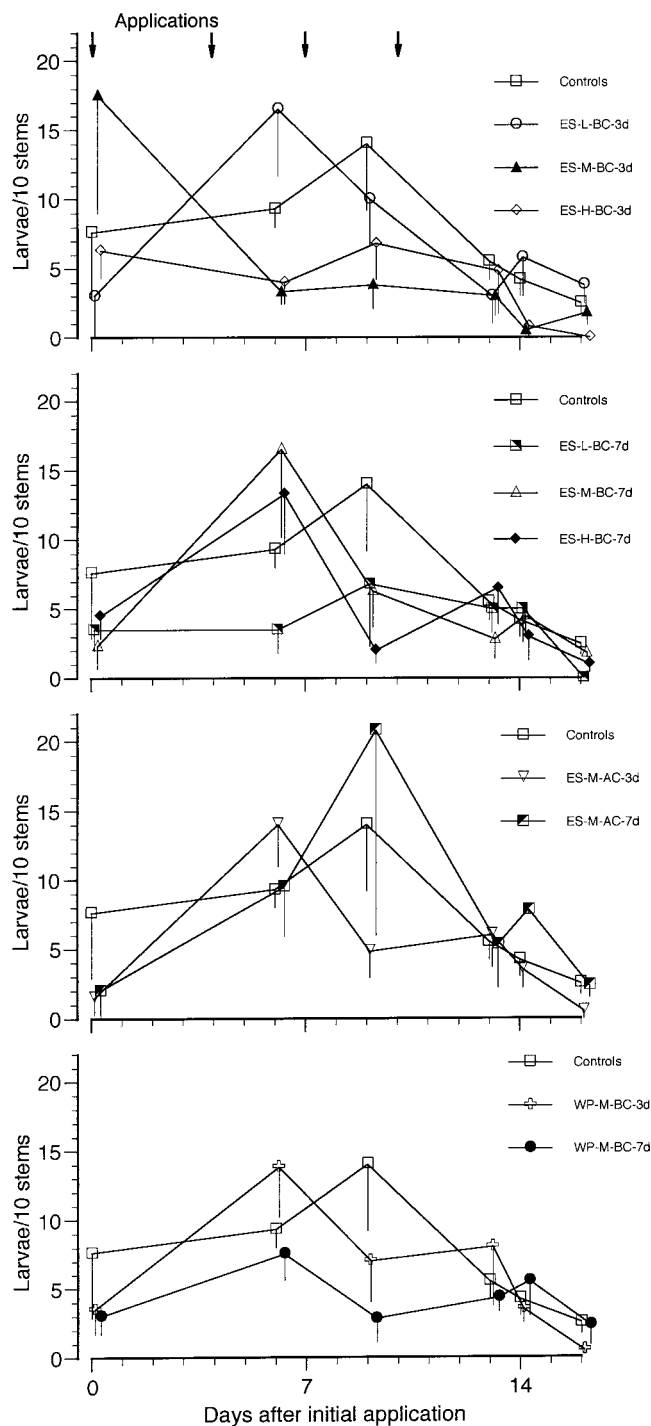


FIG. 2. Trends in Colorado potato beetle larval populations during a program of multiple spray applications of *Beauveria bassiana* conidia during a 1997 field trial; vertical lines represent standard errors of means ($n = 4$). Four applications were made at 3- to 4-day intervals on 1, 5, 8, and 11 July; two weekly applications were made on 1 and 8 July. Explanation of treatment codes is presented in Table 1.

tion between treatment and date ($P = 0.18$). As with the ES formulation, 19% of the total conidia from the WP application were deposited on the ventral leaf surfaces.

Applied from below the crop canopy at 3-day intervals, the medium and high rates of the ES formulation caused significant 54–65% reductions in larval populations compared to the combined controls, whereas the low rate was ineffective (Table 2 and Fig. 2). In contrast, following the weekly applications of the three rates, only the level of control in the low-rate treatment (42%) was significant ($P < 0.05$); the medium and high rates produced only 10–27% control (Table 2). Applications of the medium rate from above the crop canopy at 3- or 7-day intervals produced no significant reductions in larval populations (0–18%). Population

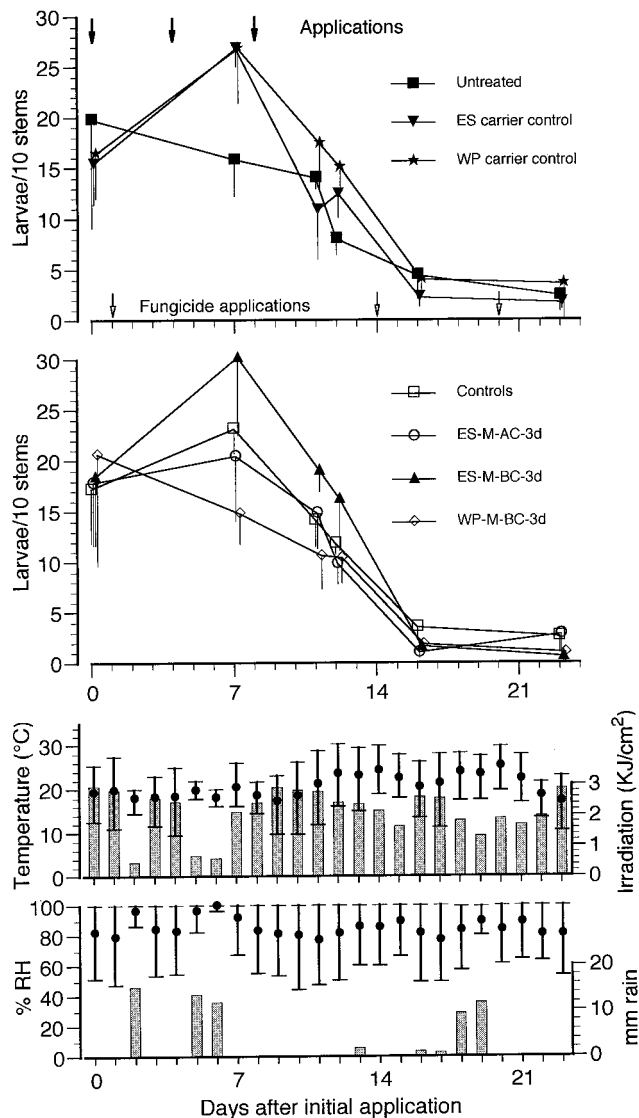


FIG. 3. Trends in Colorado potato beetle larval populations during a program of multiple spray applications of *Beauveria bassiana* conidia during a 1998 field trial; vertical lines represent standard errors of means ($n = 5$). Environmental data include temperature and relative humidity (daily means, maxima, and minima) and daily rainfall and solar irradiation. The initial application was made on 1 July. Explanation of treatment codes is presented in Table 1.

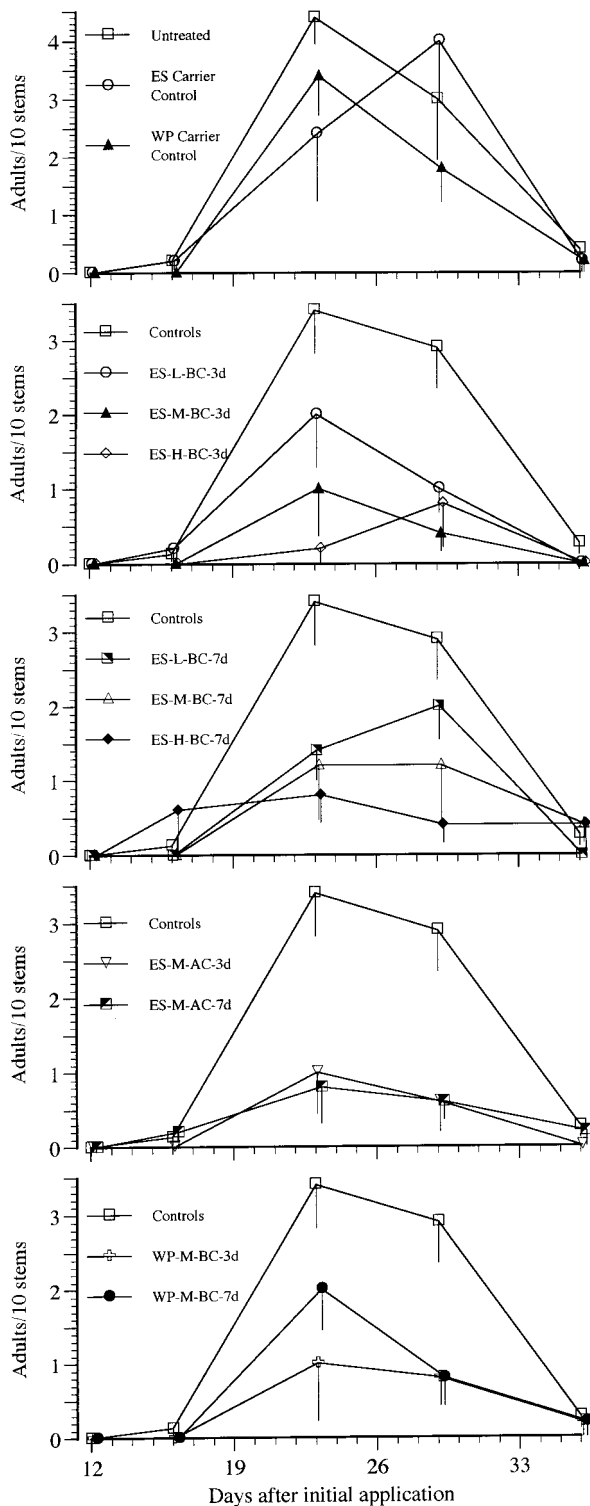


FIG. 4. Trends in Colorado potato beetle adult populations following a program of multiple spray applications of *Beauveria bassiana* conidia during a 1998 field trial; vertical lines represent standard errors of means ($n = 5$). Three applications were made at 4-day intervals on days 0, 4, and 8 after initial application; two weekly applications were made on days 0 and 8 after initial application. Explanation of treatment codes is presented in Table 1.

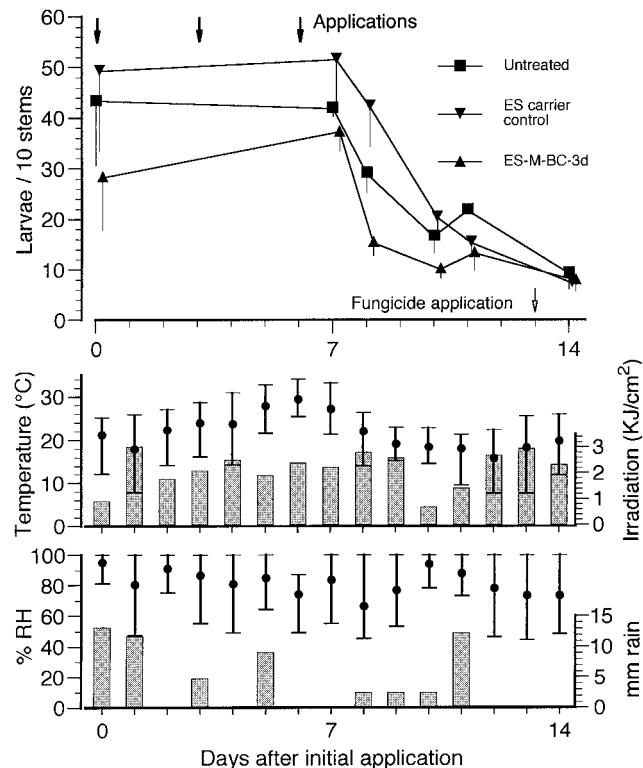


FIG. 5. Trends in Colorado potato beetle larval populations during a program of multiple spray applications of conidia of *Beauveria bassiana* during a 1999 field trial; vertical lines represent standard errors of means ($n = 6$). Environmental data include temperature and relative humidity (daily means, maxima, and minima) and daily rainfall and solar irradiation. The initial application was made on 29 June. Explanation of treatment codes is presented in Table 1.

reductions due to applications of the WP formulation at 3- or 7-day intervals also were low (8–37%) and not statistically significant. Significant reductions in defoliation were recorded in only two treatments, the medium and high rates of the ES formulation applied from below canopy at 3-day intervals (Table 2).

1998 Results

As in 1997, the initial application was made on 1 July. However, in this case, daily, heavy rains delayed application for 6-days after initial egg hatch, and by 1 July, the larval population was 84.1% late instars (primarily third instars).

Total (dorsal+ventral surface) conidia deposited by the below- versus above-canopy sprays of the ES formulation at the medium rate averaged 1089 and 990 conidia/mm², respectively, over the three spray dates (Table 3). Total conidia deposition did not differ significantly between the treatments or among the spray dates [$F(1, 6) = 0.76$; $P = 0.42$], and there was no treatment \times date interaction ($P = 0.12$). Removal of the central (downward directed) nozzle from the boom with the drop tubes used in 1997 resulted in a 10%

TABLE 2

Colorado Potato Beetle Larval Populations and Levels of Defoliation in Plots Receiving Various *Beauveria bassiana* Treatments during a 1997 Field Test in Freeville, New York

| Treatment ^a | Larvae/10 stems (% Population reduction) ^b | ANOVA statistics ^c | Defoliation (%) | ANOVA statistics ^c |
|------------------------|-------------------------------------------------------|-------------------------------|-----------------|-------------------------------|
| Untreated control | 6.5 ± 1.8 | | 7.3 ± 1.3 | |
| ES carrier control | 6.4 ± 1.1 | | 7.2 ± 2.8 | |
| WP carrier control | 8.3 ± 1.7 | | 10.0 ± 2.9 | |
| Combined controls | 7.1 ± 0.9 | $F = 0.57; P > 0.50$ | 8.2 ± 1.3 | $F = 2.25; P > 0.10$ |
| ES-L-BC-3d | 7.8 ± 0.9 (0) | — | 8.3 ± 2.1 | — |
| ES-M-BC-3d | 2.5 ± 0.4 (65) | $q = 3.86; P < 0.01$ | 2.7 ± 1.0 | $q = 5.85; P < 0.01$ |
| ES-H-BC-3d | 3.3 ± 0.2 (54) | $q = 2.67; P < 0.05$ | 5.2 ± 2.2 | $q = 2.75; P < 0.05$ |
| ES-L-BC-7d | 4.1 ± 1.5 (42) | $q = 2.65; P < 0.05$ | 6.3 ± 1.6 | $q = 1.27; P > 0.05$ |
| ES-M-BC-7d | 6.4 ± 1.7 (10) | $q = 0.61; P > 0.05$ | 7.5 ± 1.8 | $q = 0.30; P > 0.05$ |
| ES-H-BC-7d | 5.2 ± 0.8 (27) | $q = 0.98; P > 0.05$ | 6.2 ± 2.3 | $q = 1.65; P > 0.05$ |
| WP-M-BC-3d | 6.5 ± 1.3 (8) | $q = 0.17; P > 0.05$ | 7.7 ± 2.5 | $q = 0.55; P > 0.05$ |
| WP-M-BC-7d | 4.5 ± 0.9 (37) | $q = 1.61; P > 0.05$ | 6.2 ± 2.0 | $q = 1.57; P > 0.05$ |
| ES-M-AC-3d | 5.8 ± 0.6 (18) | $q = 0.46; P > 0.05$ | 6.0 ± 2.1 | $q = 1.82; P > 0.05$ |
| ES-M-AC-7d | 9.1 ± 3.1 (0) | — | 6.8 ± 1.5 | $q = 0.85; P > 0.05$ |

^a Complete explanation of treatment codes is presented under Table 1.

^b Mean larvae (± standard error; combined controls, $n = 12$; treatments, $n = 4$) per 10 stems per sample date from five consecutive samples collected on days 6, 9, 13, 14, and 16 after the initial application; numbers in parentheses indicate percent population reductions relative to the combined controls.

^c ANOVA F test ($df = 2, 36$) comparing untreated and spray-carrier controls; Dunnett's q test (one-tailed hypothesis; ($df = 38, 11$)) comparing each fungus treatment to the combined controls.

increase in deposition on the ventral leaf surfaces (from 19% observed in 1997 to 29%). As observed in 1997, the above-canopy spray deposited very few conidia (only 1%) on the ventral leaf surfaces. Also as observed in 1997, conidial deposits from the ES versus WP medium-rate applications (monitored only on ven-

tral leaf surfaces) did not differ significantly [$F(1, 6) = 0.2; P = 0.65$]. Conidial samples collected from plots sprayed from below canopy with the low, medium, and high (1.25, 2.5, and 5×10^{13} conidia/ha) rates of *B. bassiana* indicated ventral leaf surface deposits of 189, 316, and 430 conidia/mm² (Table 3).

TABLE 3

Numbers of Conidia^a Deposited on Coverslips Attached to the Dorsal and Ventral Surfaces of Potato Leaves During Spray Applications^b of *Beauveria bassiana* in Three Field Tests

| Application date | ES-L-BC | ES-M-BC | | ES-H-BC | ES-M-AC | | WP-M-BC | |
|------------------|-----------|------------|-----------|-----------|------------|----------|------------|-----------|
| | Ventral | Dorsal | Ventral | Ventral | Dorsal | Ventral | Dorsal | Ventral |
| 1997 | | | | | | | | |
| 1 July | — | 1192 ± 135 | 398 ± 80 | — | 904 ± 112 | 77 ± 47 | 754 ± 121 | 247 ± 70 |
| 8 July | — | 1218 ± 207 | 290 ± 67 | — | 1199 ± 141 | 2 ± 1 | 1253 ± 164 | 300 ± 72 |
| 11 July | — | 1001 ± 105 | 89 ± 25 | — | 1058 ± 154 | 5 ± 3 | 926 ± 129 | 140 ± 34 |
| Means | | 1137 ± 68 | 259 ± 91 | | 1054 ± 85 | 28 ± 25 | 978 ± 146 | 229 ± 47 |
| 1998 | | | | | | | | |
| 1 July | 387 ± 149 | 668 ± 77 | 558 ± 123 | 490 ± 96 | 858 ± 64 | 9 ± 3 | — | 560 ± 105 |
| 5 July | 114 ± 30 | 614 ± 99 | 156 ± 52 | 272 ± 135 | 960 ± 75 | 11 ± 6 | — | 192 ± 109 |
| 9 July | 65 ± 25 | 1037 ± 196 | 233 ± 51 | 529 ± 138 | 1122 ± 144 | 10 ± 4 | — | 161 ± 43 |
| Means | 189 ± 100 | 773 ± 133 | 316 ± 123 | 430 ± 80 | 980 ± 77 | 10 ± 1 | | 304 ± 128 |
| 1999 | | | | | | | | |
| 29 June | — | 867 ± 172 | 746 ± 84 | — | 1420 ± 124 | 81 ± 23 | 697 ± 128 | 725 ± 151 |
| 2 July | — | 790 ± 135 | 709 ± 125 | — | 1175 ± 202 | 195 ± 74 | 432 ± 106 | 518 ± 102 |
| 6 July | — | 536 ± 125 | 490 ± 97 | — | 862 ± 112 | 63 ± 23 | 524 ± 118 | 736 ± 133 |
| Means | | 731 ± 100 | 648 ± 80 | | 1152 ± 161 | 113 ± 41 | 551 ± 78 | 660 ± 71 |

^a Mean conidia per square millimeter ± standard error ($n \approx 25$ coverslips).

^b Explanation of treatment codes is presented under Table 1.

TABLE 4

Colorado Potato Beetle First-Generation Adult Populations in Plots Receiving Various *Beauveria bassiana* Treatments during a 1998 Field Test in Freeville, New York

| Treatment ^a | Adults/10 stems (% population reduction) ^b | ANOVA statistics ^c |
|------------------------|-------------------------------------------------------|-------------------------------|
| Untreated controls | 3.7 ± 0.4 | |
| ES carrier control | 3.2 ± 1.0 | |
| WP carrier control | 2.6 ± 0.5 | |
| Combined controls | 3.2 ± 0.4 | $F = 0.53; P > 0.50$ |
| ES-L-BC-3d | 1.5 ± 0.5 (53) | $q = 2.63; P < 0.05$ |
| ES-M-BC-3d | 0.7 ± 0.4 (78) | $q = 4.68; P < 0.01$ |
| ES-H-BC-3d | 0.5 ± 0.3 (84) | $q = 5.16; P < 0.01$ |
| ES-L-BC-7d | 1.7 ± 0.4 (47) | $q = 2.14; P > 0.05$ |
| ES-M-BC-7d | 1.2 ± 0.3 (63) | $q = 3.11; P < 0.05$ |
| ES-H-BC-7d | 0.6 ± 0.2 (81) | $q = 4.68; P < 0.01$ |
| WP-M-BC-3d | 0.9 ± 0.5 (72) | $q = 4.27; P < 0.01$ |
| WP-M-BC-7d | 1.4 ± 0.4 (56) | $q = 2.69; P < 0.05$ |
| ES-M-AC-3d | 0.8 ± 0.4 (75) | $q = 4.45; P < 0.01$ |
| ES-M-AC-7d | 0.7 ± 0.3 (78) | $q = 4.45; P < 0.01$ |

^a Complete explanation of treatment codes is presented under Table 1.

^b Mean larvae (±standard error; combined controls, $n = 15$; treatments, $n = 5$) per 10 stems per sample date from two consecutive samples collected on days 23 and 29 after the initial application; numbers in parentheses indicate percent population reductions relative to the combined controls.

^c ANOVA F test ($df = 2, 48$) comparing untreated and spray-carrier controls; Dunnet's q test (one-tailed hypothesis; ($df = 50, 11$)) comparing each fungus treatment to the combined controls.

No significant differences in larval populations were noted among all treatments and controls on day 7 [$F(10, 40) = 1.3; P = 0.28$] or on days 7–12 combined [$F(10, 40) = 0.6; P = 0.57$]. Significant differences were detected among samples collected on day 16; however, by this date, approximately 85% of the population in the controls had completed larval development and

entered the soil to pupate (Fig. 3). The low-density larval populations inflicted minor, statistically insignificant damage (7–12% defoliation) in all treatments. Due to the detection of only minimal treatment effects, sampling was extended and ultimately revealed that the foliar applications applied against the late-instar larvae had highly significant impacts on the numbers of emerging first-generation adults (Fig. 4). All treatments, except the low rate applied at 7-day intervals, resulted in significant reductions (53–84%) in adult populations (Table 4).

1999 Results

Planting was conducted under severe drought conditions. Uneven seed sprouting and emergence produced poor stands of variable age in large areas of the field, making it impossible to apply all treatments of the previous years. In addition, large numbers of adult beetles caused severe damage to plants emerging in isolation, making it necessary to reduce adult beetle pressure by hand picking. At the time of application, the population comprised a broad range of developmental stages, but 90.8% of the larvae were still first and second instars.

Conidial samples indicated similar total depositions [$F(1, 6) = 0.4; P = 0.56$] for the ES medium rate applied from below versus above canopy (1379 versus 1265 conidia/mm²); 47 and 10% of the total conidia were deposited on the ventral leaf surfaces by the respective treatments (Table 3). Application of the WP formulation at the medium rate from below the crop canopy produced total deposits equal to the comparable ES treatment (1211 versus 1379 conidia/mm²); 55% of the total conidia were deposited on the ventral leaf surfaces.

TABLE 5

Colorado Potato Beetle Larval Populations and Levels of Damage in Plots Receiving Various *Beauveria bassiana* Treatments during a 1999 Field Trial in Freeville, New York

| Treatment ^a | Larvae/10 stems (% population reduction) ^b | ANOVA statistics ^c | Defoliation (%) | ANOVA statistics ^c | Yield (kg/row meter) | ANOVA statistics ^c |
|------------------------|-------------------------------------------------------|-------------------------------|-----------------|-------------------------------|----------------------|-------------------------------|
| Untreated control | 19.1 ± 1.5 | | 34.8 ± 8.0 | | 0.51 ± 0.06 | |
| ES carrier control | 21.3 ± 2.3 | | 29.2 ± 3.9 | | 0.55 ± 0.09 | |
| Combined controls | 20.2 ± 1.3 | $F = 0.57; P > 0.50$ | 32.0 ± 4.3 | $F = 0.46; P > 0.50$ | 0.53 ± 0.08 | $F = 0.53; P > 0.50$ |
| ES-M-BC-3d | 11.6 ± 1.3 (43) | $q = 3.86; P < 0.01$ | 11.8 ± 2.2 | $q = 3.71; P < 0.01$ | 0.70 ± 0.11 | $q = 3.11; P < 0.01$ |
| ES-M-BC-7d | 17.4 ± 2.6 (14) | $q = 0.61; P > 0.05$ | 19.8 ± 4.9 | $q = 2.18; P > 0.05$ | 0.62 ± 0.11 | $q = 1.65; P > 0.05$ |
| WP-M-BC-3d | 16.1 ± 3.1 (20) | $q = 0.17; P > 0.05$ | 11.5 ± 3.2 | $q = 3.92; P < 0.01$ | 0.60 ± 0.12 | $q = 1.30; P > 0.05$ |
| ES-M-AC-3d | 22.8 ± 4.4 (0) | $q = 0.46; P > 0.05$ | 26.2 ± 5.5 | $q = 0.98; P > 0.05$ | 0.55 ± 0.10 | $q = 0.29; P > 0.05$ |

^a Explanation of treatment codes is presented under Table 1.

^b Mean larvae (±standard error; combined controls, $n = 12$; treatments, $n = 6$) per 10 stems per sample date from four consecutive samples collected on days 8, 10, 11, and 14 after the initial application; numbers in parentheses indicate percent population reductions relative to the combined controls.

^c ANOVA F test ($df = 2, 25$) comparing untreated and spray-carrier controls; Dunnet's q test (one-tailed hypothesis; ($df = 26, 5$)) comparing each fungus treatment to the combined controls.

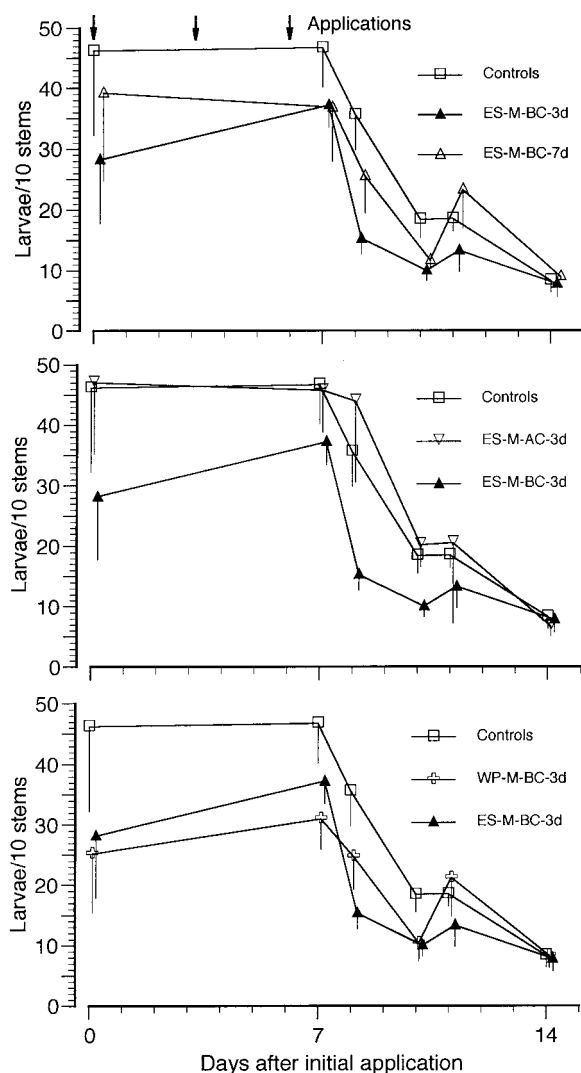


FIG. 6. Trends in Colorado potato beetle larval populations during a program of multiple spray applications of conidia of *Beauveria bassiana* during a 1999 field trial; vertical lines represent standard errors of means ($n = 6$). Three applications were made at 3-day intervals on 29 June and 2 and 5 July; two weekly applications were made on 29 June and 5 July. Explanation of treatment codes is presented in Table 1.

Of the four treatments tested (all employing the medium rate), only applications of the ES formulation from below canopy at 3-day intervals produced a significant reduction in the larval population (Fig. 6 and Table 5). Control achieved, however, was poor (43%). The WP formulation applied in the same manner produced only a 20% reduction in larval populations, a level not significantly different from the controls ($P > 0.10$). Application of the ES formulation on the same schedule, but from above the canopy, had no measurable effect on the larval population. Defoliation was significantly reduced from 32 to 12% by applications of both the WP and ES formulations from below canopy at 3-day intervals; however, a significant increase in yield

(from 0.53 to 0.7 kg/row meter) was recorded only in the ES treatment (Table 5).

DISCUSSION

None of the foliar-spray treatments applied during this study provided a high level of larval control over any significant part of the growing season. Clearly, the use of *B. bassiana* strain GHA as a stand-alone product to protect crops from defoliation by first-generation Colorado potato beetle larvae cannot be recommended on the basis of these results, which are in accord with the findings of other studies (see Hajek *et al.*, 1987). On the other hand, some researchers have obtained better results from similar treatments applied with portable spray equipment (e.g., Poprawski *et al.*, 1997). The unexpectedly poor performance of even the most aggressive treatments in this study (applications at a high rate from below canopy at 3- to 4-day intervals) is difficult to explain. Nevertheless, the results indicated that manipulation of various application parameters can significantly influence the efficacy of *B. bassiana* and identified potentially useful avenues for further research. The relatively high rates of reduction of first-generation adult populations achieved by treatment of late-instar larvae during the 1998 season is particularly interesting and warrants additional study.

Application Rate

Results of the 1997 and 1998 tests indicated that the low rate was less effective than the two higher rates. With respect to the 1997 results, the complete lack of efficacy of the low rate applied from below canopy at 3-day intervals and the relatively high level of efficacy associated with the 7-day, low-rate applications appear to represent anomalous extremes (Table 2). Considering the clear dose responses in the adult population data (Table 4) and the generally unacceptable levels of control achieved overall, the more economical low rate cannot be recommended.

The medium and high rates generally produced similar levels of control (Tables 2 and 4). This may be explained by the unexpected finding in 1998 of a much smaller than expected difference between conidial depositions produced by these application rates [$F(1, 10) = 0.4$; $P = 0.56$]. A possible explanation may involve what we observed to be a strong tendency of the emulsified oil droplets to rise to the top of the mixing bottles and coalesce into a ring deposit that adhered strongly to the polyethylene plastic. This deposit of conidia in oil formed rapidly while the suspensions were in queue for use and increased with increasing concentration of the ES formulation. Once formed, it was impossible to resuspend this material by shaking. In fact, excessive shaking resulted in coalescence of oil droplets and further destabilization of the emulsion

(segregation of the oil and water components). Likely, in most cases, this problem can be overcome by providing constant, gentle agitation (readily achieved with commercial spray equipment, e.g., with recirculation agitators). Nevertheless, such problems underscore a need for caution when using oil-in-water emulsions as carriers for *B. bassiana* conidia and call for further improvements in oil formulations.

Spray Interval

Results from the three field seasons support the prediction of the Larkin *et al.* model (see Galaini, 1984) that weekly sprays would not be adequate to protect the crop from defoliation by beetle larvae. Among the 11 weekly treatments applied during the study (Table 1), only one (the 1997 ES low dose applied from below canopy) produced a significant population reduction, and this result was likely spurious, as the comparable medium- and high-rate treatments were ineffective (Table 2). Surprisingly, however, the two sprays applied at 7-day intervals in 1998 were nearly as effective in reducing the first-generation adult population as three sprays applied at 3-day intervals (Table 4). This unexpected result may have important implications with respect to development of beetle control strategies and is discussed in greater detail below.

Sprayer Configuration

Use of drop-tube boom configurations to target first-instar larvae feeding on the ventral surfaces of the foliage and deposit conidia in a microhabitat protected from fungicidal solar radiation proved highly beneficial. Of the five treatments that produced significant reduction in the 1997 and 1999 larval populations, all were made from below canopy (Tables 2 and 5). These results indicate that additional research in this area might be productive.

The only instances of significant control achieved with above-canopy sprays were recorded in 1998 when treatments were initiated against a population of late instars, and in this case, treatment effects were detected only after adult emergence (Table 4). This result suggests either that the mature larvae or pupae acquired a lethal dose of conidia from the soil or that the late-instar larvae were effectively targeted by the above-canopy sprays. The latter explanation is supported by the fact that the levels of adult control were equivalent in the comparable above- versus below-canopy spray treatments (75 vs 78%, respectively, from sprays applied at 3-day intervals and 78 vs 81%, respectively, from sprays applied 7 days apart) (Table 4). Late-instar larvae typically feed in the upper canopy, often in fully exposed positions on the edges or upper surfaces of the leaves. Resolution of this question will require further investigation.

Recent bioassays with strain GHA against second-instar Colorado potato beetle larvae from a laboratory colony (maintained by the New Jersey Department of Agriculture, West Trenton, NJ) indicated a mean LC_{50} of 121 conidia/mm² ($SE = \pm 38.9$; $n = 6$) with a mean probit regression slope of 1.5 (J. D. Vandenberg and M. H. Griggs, personal communication). In the 1997 field tests, the multiple, below-canopy applications of the ES formulation deposited an average of 259 conidia/mm² on the ventral leaf surfaces (Table 3) and achieved 65% control (Table 2). This rate is notably close to the LC_{65} of 217 conidia/mm² predicted by the probit model from the single-application laboratory assays and suggests either that effects of the multiple applications in the field were not cumulative or that the field LC_{50} was much greater than the laboratory LC_{50} . The numbers of conidia deposited on ventral leaf surfaces in 1999 were substantially higher (mean 648 conidia/mm²) (Table 3), likely due to the smaller size of the drought-stressed plants. Yet, in this case, control achieved was only 43% (Table 5). This supports a hypothesis of fungal inhibition by adverse environmental conditions discussed below.

Fungal Formulation

The ES formulation provided greater control of larvae than the WP (Tables 2, 4, and 5). In 1997 and 1999, larval populations were reduced 65 and 43%, respectively, by the ES treatments versus only 8 and 20% by the comparable WP treatments. Lower efficacy of the WP relative to the ES may be a consequence of the two formulations differing in rainfastness. Significant rain fell during the late afternoon immediately following two of the 1997 applications (days 0 and 7, Fig. 1), and rainfall was also recorded at various times following applications in 1998 and 1999 (Figs. 3 and 5). This hypothesis is supported by recent findings that *B. bassiana* conidia formulated in some emulsifiable oils exhibited greater rainfastness than WP-formulated conidia (Inglis *et al.*, 1999), and by the fact that a number of field studies conducted with grasshoppers and whiteflies under arid conditions have failed to show significantly greater efficacy of oil formulations compared to wettable powders [see reviews by Wraight and Carruthers (1999) and Wraight *et al.* (2001)].

With the exception of the 1997 results, however, the differences in control produced by the two formulations were not large. During the field season with the largest pest infestation (1999, Table 5), the WP produced only an insignificant 20% reduction in the larval population relative to the controls ($P > 0.10$). However, the ES formulation also produced only a low level of control (43%), and while this population reduction was significant ($P > 0.05$), a direct orthogonal comparison of larval numbers indicated no significant difference [$F(1, 25) = 1.37$; $P > 0.50$] between the two treat-

ments (formulations). Similarly, the reductions in numbers of first-generation adults recorded in the 1998 ES and WP formulation treatments were practically equivalent (78% vs 72% in the 3-day treatments and 63% vs 56% in the 7-day treatments, respectively) (Table 4).

Other Factors

The formulation effects discussed above suggest that rainfall following the spray applications may have been an important factor in the overall poor larvicidal efficacy observed in our tests. Inglis *et al.* (1995b) reported a 28–61% loss of unfertilized *B. bassiana* colony-forming units (CFU) from wheat and alfalfa foliage exposed to simulated rain, and Inglis *et al.* (1999) observed losses of 0–45% of *B. bassiana* CFU formulated as Mycotrol ES and a 66% loss of CFU formulated as Mycotrol 22WP from potato foliage exposed to simulated rain; the corresponding losses from Colorado potato beetle larvae were 4–14% and 40%, respectively. While the effect of rain on the persistence of the conidia applied in our tests was not measured, these findings indicate that it may have been significant with respect to both the WP and ES formulations.

At first consideration, it does not appear that extreme temperatures or relative humidities (RH) were important factors. The mean temperatures recorded over the initial 10 days of the 1997, 1998, and 1999 trials were only 18.4, 18.7, and 23.2°C, respectively; respective mean daily maxima were 23.6, 23.3, and 28.6°C (Figs. 1, 3, and 5). Mean RH over the same time periods were 86.3, 87.4, and 81.3% with mean minima of 66.9, 65.4, and 58.3%. On the other hand, the warmer conditions during 1999 (when fungal efficacy was lowest) generated a greater mean vapor pressure deficit (4.51 mm Hg) during the initial 10 days than was recorded in 1997 (2.85 mm Hg) or 1998 (2.78 mm Hg). Mounting evidence also indicates that *B. bassiana* may be inhibited by temperatures commonly encountered in the field-crop environment (even in temperate climates). Long *et al.* (2000) recently observed an inverse relationship between *B. bassiana* strain GHA-induced mortality of Colorado potato beetle prepupae and temperatures ranging from 15 to 30°C. Inglis *et al.* (1996) reported substantial inhibition of *B. bassiana* strain GHA mycosis in grasshoppers allowed to bask (increasing body temperature to nearly 40°C) for only 1 h/day.

Fargues *et al.* (1997a) determined that the optimal constant temperature for radial hyphal growth of *B. bassiana* strain GHA was approximately 28°C and that growth rates relative to the maximum were reduced by 20, 60, and 99% at 30, 32, and 35°C, respectively. While 35°C represented the approximate cardinal temperature, Inglis *et al.* (1996) observed that this temperature did not significantly inhibit hyphal growth unless cul-

tures were exposed for a period of at least 4 h each day. Similarly, disease development in infected grasshoppers was affected only after exposure to 35°C for a period of 6 h/day. While the high degree of behavioral thermoregulation exhibited by grasshoppers, and the negative effects of this behavior on fungal pathogenesis (Carruthers *et al.*, 1992; Inglis *et al.*, 1996), have not been reported in potato beetles, it has been shown that, under sunny conditions, the internal body temperatures of Colorado potato beetle larvae may be elevated several degrees above ambient (May, 1981, 1982). May (1981) noted an average elevation of 3.4°C. Thus, internal body temperatures would be expected to approach the strongly inhibitory range of 32–35°C at ambient readings of approximately 30°C. Significant intervals with temperatures $\geq 30^\circ\text{C}$ were recorded prior to peak control only during the 1999 test (Fig. 5). Temperatures $>30^\circ\text{C}$ prevailed for an average of 7 h/day for the 4-day period between 3 and 6 July (days 4–7 after the initial application). Mean daily solar irradiation levels were also highest during this test (Figs. 1, 3, and 5). These conditions may have been a factor in the exceptionally low efficacy recorded in 1999 (at least having the effect of slowing fungal development and delaying mortality until after larvae entered the soil). Lacey *et al.* (1999) reported improved control of Colorado potato beetle larvae following row (canopy) closure and suggested that this "... coincided with higher humidity and increased protection from sunlight." The potato plants were stunted by drought conditions during our 1999 trial and never reached sufficient size to close the rows. This could have exacerbated the effects of what otherwise might have been only marginally inhibitory temperatures.

The fungicides most frequently applied in our tests, mancozeb and manzeb, are highly toxic to *B. bassiana* when incorporated into culture media in the laboratory (Olmert and Kenneth, 1974; Loria *et al.*, 1983), and these materials thus are potentially antagonistic when applied to the same foliage as *B. bassiana* in the field. Surprising results from a recent study indicated, however, that standard applications of mancozeb and other fungicides under field conditions had little effect on *B. bassiana* efficacy against Colorado potato beetle, at least when not applied on the same day as the *Beauveria* (Jaros-Su *et al.*, 1999). The low levels of efficacy observed in each of our trials were clearly also not solely a result of fungicide interactions. Few fungicide applications were made during the *Beauveria* spray programs. Dry conditions during the spring of 1999 reduced the risk of a late blight outbreak, and the initial fungicide application was not made until day 11 posttreatment, well after the first generation larval population had begun to decline and maximum control due to *Beauveria* had already been achieved (Fig. 3).

Pest Management Implications

The extremely rapid destructive capacity of Colorado potato beetle is an important factor limiting commercial viability of slow acting biological control agents such as *B. bassiana*. In response to this problem, use strategies have been developed that emphasize timing of initial applications to correspond with egg hatch and application of subsequent treatments at short (3- to 4-day) intervals in order to target later hatching larvae (Galaini, 1984). Poor efficacy of less frequent sprays has been attributed to many early-instar larvae escaping inoculation. This strategy is sound when the objective is rapid control of a damaging infestation. Early-instar larvae are more susceptible than later instars, at least in terms of lethal time (Blonska, 1957; Fargues, 1972), and as the 1998 results show, spray applications must target the early instars in order to kill a large proportion of the larval population in time to afford protection from defoliation and yield loss. The unexpected observation of high levels of control of first generation adults in the 1998 trial (in which the spray program was initiated against a population comprising primarily third-instar larvae) suggests that targeting late instars could be an effective strategy for long-term control of beetle populations in integrated, areawide management systems.

It has been recognized that assessing the total impact of foliar applications against larval populations requires monitoring of subterranean mortality or adult emergence (Timonin, 1939; Blonska, 1957; Lappa, 1978; Fargues *et al.*, 1980; Campbell *et al.*, 1985; Long *et al.*, 2000). However, few studies have examined the long-term control potential of applications against early- versus late-instar larvae, and published results have been mixed. We, unfortunately, did not sample adult populations derived from populations of larvae treated as early instars (1997 and 1999). Blonska (1957) treated larvae of each of the four instars on individual plants in the field and monitored mortality through adult emergence; results indicated that early instars were more susceptible to lethal infection than late instars. In contrast, Fargues (1972), reported that the four larval instars were equally susceptible to infection by *B. bassiana* and that susceptibility was inversely related to instar only in terms of lethal time.

Data from a number of studies indicate that good control of first generation adults was achieved even though applications initiated against early-instar larvae did not provide high levels of control prior to larval maturation (Lappa, 1978; Roberts *et al.*, 1981; Anderson *et al.*, 1988; Lacey *et al.*, 1999). On the other hand, Campbell *et al.* (1985) initiated applications at first appearance of third instars, and subsequent control of first generation adults was poor (a posttreatment population of approximately three mature larvae/stem gave rise to a population of two adults/stem).

Observations of *Beauveria* affecting soil stages of the beetle both under natural conditions and as the result of foliar spray programs has stimulated considerable investigation of the potential of soil applications. Results, however have been as inconsistent as those from foliar sprays (Watt and LeBrun, 1984; Cantwell *et al.*, 1986; Gaugler *et al.*, 1989). The difficulty and expense of achieving effective titers of infectious units throughout the entire soil matrix (see Wraight and Carruthers, 1999) and negative interactions with antagonistic microbes and other soil factors (Lingg and Donaldson, 1981; Groden and Lockwood, 1991) are important constraints. The 1998 results are from only a single trial, but nevertheless suggest that more efficient and consistent control of subterranean stages might be achievable by targeting the epigeal populations of late-instar larvae. Large larvae feeding in the crop canopy are more easily targeted than small larvae feeding on the ventral leaf surfaces. First-instar larvae feeding on the undersides of leaves near the ground (a common oviposition site) and prepupae or pupae residing in the soil are especially difficult targets. The observation that the above-canopy sprays reduced the populations of first generation adults as effectively as the less conventional and more difficult below-canopy sprays (Table 4) is especially noteworthy in this context.

Results of these tests reveal that a broad range of readily manipulable factors can influence the effectiveness of *B. bassiana* against Colorado potato beetle. This, in turn, indicates a great potential for formulation and application technologies to continue providing for incremental improvements in mycoinsecticide efficacy and suggests that continued advances on numerous fronts may ultimately lead to products that are more economically competitive with the chemical insecticides currently used to control this key pest.

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